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STATE-OF-THE-ART PAPERS

Relevant Issues in the Pathology and Pathobiology of Pulmonary Hypertension

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Knowledge of the pathobiology of pulmonary hypertension (PH) continues to accelerate. However, fundamental gaps remain in our understanding of the underlying pathological changes in pulmonary arteries and veins in the different forms of this syndrome. Although PH primarily affects the arteries, venous disease is increasingly recognized as an important entity. Moreover, prognosis in PH is determined largely by the status of the right ventricle, rather than the levels of pulmonary artery pressures. It is increasingly clear that although vasospasm plays a role, PH is an obstructive lung panvasculopathy. Disordered metabolism and mitochondrial structure, inflammation, and dysregulation of growth factors lead to a proliferative, apoptosis-resistant state. These abnormalities may be acquired, genetically mediated as a result of mutations in bone morphogenetic protein receptor-2 or activin-like kinase-1, or epigenetically inherited (as a result of epigenetic silencing of genes such as superoxide dismutase-2). There is a pressing need to better understand how the pathobiology leads to severe disease in some patients versus mild PH in others. Recent recognition of a potential role of acquired abnormalities of mitochondrial metabolism in the right ventricular myocytes and pulmonary vascular cells suggests new therapeutic approaches, diagnostic modalities, and biomarkers. Finally, dissection of the role of pulmonary inflammation in the initiation and promotion of PH has revealed a complex yet fascinating interplay with pulmonary vascular remodeling, promising to lead to novel therapeutics and diagnostics. Emerging concepts are also relevant to the pathobiology of PH, including a role for bone marrow and circulating progenitor cells and microribonucleic acids. Continued interest in the interface of the genetic basis of PH and cellular and molecular pathogenetic links should further expand our understanding of the disease. (J Am Coll Cardiol 2013;62:D4–12) © 2013 by the American College of Cardiology Foundation

The specific field of pathobiology of pulmonary hypertension (PH) has undergone impressive growth since the last world meeting in Dana Point, California, in 2008 (1,2). Building on a remarkable list of significant accomplishments realized in the past 110 years (3), several important paradigms have taken center stage, including the role of metabolic reprogramming and inflammation, with promising insights that may lead to novel therapeutic and diagnostic targets. Notwithstanding this

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remarkable progress, central questions remain related to the fundamental aspects of pulmonary vascular pathology in PH.

When choosing the particular areas of emphasis in the context of the World Symposium, the Pathology and Pathobiology Working Group opted to continue focusing on the pathologic alterations of pulmonary veins in PH, largely inspired by the pressing questions in this particular area that arose from the last world meeting (1). This was complemented by 3 additional topics, due to their timeliness and overall importance: the molecular determinants of mild versus severe PH; the role of metabolic reprogramming underlying cellular responses in pulmonary vascular disease; and recent insights into the role of inflammation as a trigger and modifier of PH. Furthermore, many pathogenetic processes operational in mild disease appear to have roles in more severe PH, but how molecular processes determine the progression and ultimately the severity of the disease remains unclear. To start addressing this complex yet central question—perhaps one of the most vexing challenges in the field—the integration of pathology and pathogenetic mechanisms should provide insights into this topic in the years to come.

The group members acknowledge that the chosen discussion topics did not allow for an all-inclusive dissection of established and emerging areas of investigation. These include, among others, specific signaling pathways shown to affect pulmonary vasoconstriction and remodeling (such as epidermal growth factor [4], fibroblast growth factor [5], platelet-derived growth factor [6,7], and transforming growth factor-β [8,9]) or are protective (such as bone morphogenetic proteins), and evolving knowledge on the involvement of pathogenic (10) or protective (11) micro-ribonucleic acids in pulmonary arterial hypertension (PAH). In addition, emerging areas of investigation, including the potential role of progenitor or stem cells in pulmonary vascular remodeling (12), were not addressed. Notwithstanding these limitations, when fitting, we emphasize the potential role of circulating cells derived from the bone marrow, as documented in recent human studies (13).

Does the Pulmonary Venous System Play an Important Role in PAH and to What Extent Are PAH and PVOD Part of the Same Spectrum of Disease?

It is apparent that the different forms of PH present with either a predominance of pulmonary arterial remodeling or vein remodeling or a variable contribution of both. Paradigmatic of the former is idiopathic pulmonary arterial hypertension (IPAH), whereas pure pulmonary veno-occlusive disease and PH due to left heart dysfunction are characterized predominantly by venous remodeling. Virtually all forms of PH, including thromboembolic PH, sarcoid PH, and those caused by interstitial lung disease and hypoxia, may involve elements of both arterial and venous remodeling. However, more precise documentation, including morphometric analysis of pulmonary vein remodeling, is still lacking in most of these conditions.

Abbreviations and Acronyms

ALK = activin receptor-like kinase
BMPR2 = bone morphogenetic protein type II receptor
DC = dendritic cell
ER = endoplasmic reticulum
HIF = hypoxia inducible factor
IPAH = idiopathic pulmonary arterial hypertension
LHF = left heart failure
PAH = pulmonary arterial hypertension
PH = pulmonary hypertension

The difficulty in studying the pathology of pulmonary venous remodeling is compounded by the lack of distinct molecular markers that allow for their identification (as compared with the pulmonary arterial bed) when immersed in alveolar tissue. Normal veins can be recognized by the lack of a double elastic lamina and relatively thin muscular media; however, when remodeled, pulmonary veins become "arterialized," making their distinction from pulmonary arteries more difficult. The only distinctive feature, which is often not present in biopsies, is the presence of pulmonary veins in interlobular septae. Moreover, molecular markers of veins, such as the ephrin B4 receptor, are often difficult to identify in human (and rat) lungs. The venous coaxial structure includes layers of cardiomyocytes arrayed externally around a subendothelial layer of typical smooth muscle cells, thus forming sphincter-like structures. In disease states, this cardiac muscle layer extends further into the lung, and its role in Group 2 PH merits investigation.

Given the limitations in recognizing veins histologically, most studies rely on their identification in selected regions of explant specimens. In subsets of PAH patients with scleroderma-associated PAH, pulmonary veno-occlusive disease-like remodeling may predominate over arterial remodeling. The venous remodeling, if present, entails a worse prognosis associated with decreased 6 min-walk distance, partial pressure of oxygen in the blood, and diffusion capacity (14). However, in a recent pathologic study of PAH, including lungs with scleroderma-associated PAH, no correlations between vein remodeling and intima and media thickness in arteries could be identified (15).

Recent studies have highlighted the pattern of pulmonary vein remodeling in patients with left heart failure (LHF). Lung samples obtained from patients with LHF placed on a ventricular assist device showed increased pulmonary vein and artery media thickening. Of interest, some patients who improved on the assist device and underwent repeat lung biopsy had a significant improvement of pulmonary vein remodeling (16). The molecular drivers of these processes remain largely unknown.

We continue to lack the availability of unique molecular markers that allow the specific identification of pulmonary veins. Consequently, the prevalence of venous pathology in the various forms of PH remains unknown. This is despite the potential for deleterious effects of prostacyclin and agents that increase bioavailability of nitric oxide in the context of elevated capillary pressures. Finally, little is known about pathogenetic pathways involved in pulmonary vein remodeling and how they correlate with arterial remodeling

in both LHF and in forms of PH with pre-capillary predominance.

Are There Distinct Pathways in Vascular Cells in Mild Versus Severe PH?

Although PH is defined as a mean pulmonary arterial pressure at right heart catheterization >25 mm Hg with a normal pulmonary capillary wedge pressure, it is well known that, on the basis of clinical and hemodynamic findings, PH can range from mild to severe, even in the presence of the same stimulus (e.g., hypoxia). This spectrum of severity raises several critical pathogenetic and clinical questions in terms of the distinctive features that differentiate the severity stages.

Increased pulmonary artery pressures occur due to sustained vasoconstriction, excessive pulmonary vascular remodeling, and in situ thrombosis, which ultimately lead to increased pulmonary vascular resistance. However, the factors responsible for the aggravation or acceleration of PH remain poorly defined (Fig. 1). The contributing factors likely involve accumulation of multiple events on a background of genetic predisposition. These factors involve the action of vasoconstrictive and pro-remodeling processes, including the action of inflammatory, procoagulant, anti-apoptotic, and autoimmune mediators, cell–cell and cell–matrix interactions, and environmental factors over time (Fig. 2) (17). Although the pulmonary artery bed appears unreactive to vasodilators in advanced disease, vasoreactivity and remodeling possibly interact in disease evolution (18).

Whether the severity of pulmonary vascular disease involves a constellation of pathobiological processes or is defined pathologically, with the hallmark finding of reduction of the pulmonary vascular lumen, remains unclear. The definition of the severity of PH on the basis of histopathological findings is complicated by the lack information on what constitutes "normal." Surprisingly, recent analysis of unused donor "control" lungs revealed substantial neointimal formation, inflammation, and venous changes (15), features usually judged to be "pathological." This suggests a spectrum from pristine vessels (which are observed primarily in the younger controls) to vascular changes reminiscent of PH that may be present as a function of normal aging, including inflammation and left ventricular stiffening. Because a description of the pathology in mild forms of PH is largely unavailable, it is difficult to discern whether the pathological features we observe in "controls" are similar but still less severe than in patients with "mild" PH. Perhaps a better definition of severity would also incorporate the extent of the reduction in cross-sectional area of the pulmonary vascular bed. In this summary, we confine our discussion of severity to the extent of the pulmonary vascular remodeling process, although in clinical practice, the assessment of severity will also consider the function of the right ventricle. Below, we expand on how genetic factors influence with cellular and molecular pathogenetic processes to possibly account for the severity of pulmonary vascular disease (Fig. 1).

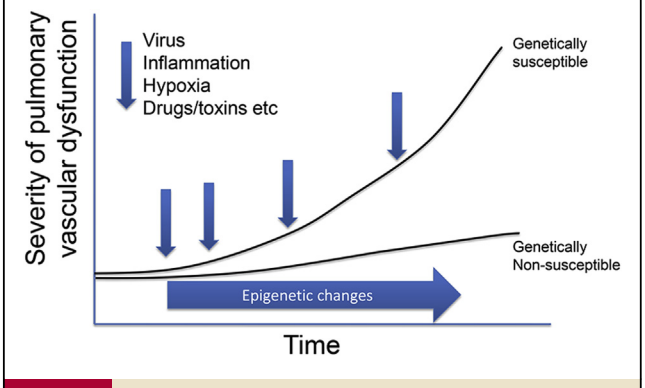

Figure 1 **Proposed Multifactorial Factors Influencing Progression of Pulmonary Hypertension**

In a suitable genetic background, the interplay of epigenetics and pathobiological injurious events may amplify the severity of the disease, often associated with more pronounced remodeling and worse clinical outcome.

Mutations in bone morphogenetic protein type II receptor (BMPR2) or activin receptor-like kinase-1 (ALK-1) are emerging as determinants of severity of PAH. Mutations in BMPR2 (19) have been reported in more than 70% of subjects with 1 or more affected relatives (heritable PAH) and 11% to 40% of those with idiopathic PAH (20,21). Mutations in several other genes have been found, including mutations in the *ALK1* gene (22), the *endoglin* gene (23), the *SMAD9* gene (24), the *Caveolin-1* gene (25), and recently, the KCNK3 gene (26). Patients with *BMPR2* or *ALK-1* mutations present with higher pulmonary vascular resistance (27). There is also evidence that these patients present and die at a younger age and with more severe disease compared with PAH patients without mutations, and they are less likely to respond acutely to vasodilators (28). Recent evidence suggests that the degree of pulmonary vascular remodeling is greater in patients with *BMPR2* mutations compared with non-*BMPR2*–related disease at the time of transplantation (4). In addition to these causal rare sequence variants, variable expressivity of PAH might be explained by genetic modifiers. For example, single nucleotide polymorphisms in several genes, such as the *angiotensin-converting enzyme* gene, the *KCNA5* gene, the *serotonin transporter* gene, and the *serotonin 5-HT2B receptor* gene have been reported to influence disease penetrance or progression. In addition, microsatellite instability of endothelial cell growth and apoptosis genes within plexiform lesions in idiopathic PAH (29), as well as somatic chromosome abnormalities (30), have been described. Most cases of PH do not have known genetic triggers; moreover, vascular cells isolated from experimental PH maintain their in vivo characteristics when placed in culture, suggesting epigenetic abnormalities. An example of such a contributing factor is the silencing of mitochondrial superoxide dismutase-2, which alters redox signaling and, therefore, activates hypoxia inducible factor (HIF)-1α, leading to the aerobic glycolysis seen in PH (31,32). However, further studies, particularly focused on

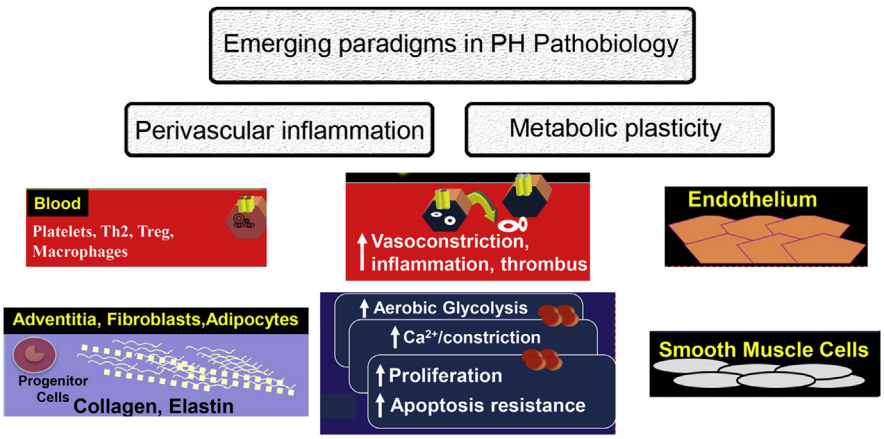

Figure 2 **Emerging Paradigms in PH Research**

Research involving the broad effects of metabolic programming of intima and media pulmonary vascular cells (endothelial and smooth muscle cells) and the immediate perivascular microenvironment. The perivascular region is dominated by fibroblasts and migrating circulating cells, including inflammatory and progenitor cells. Shown in the **center** is the impact of these factors in the intima and media of pulmonary arteries. The metabolic plasticity involves all cells involved in the pulmonary hypertension (PH) panvasculopathy and is itself modified by inflammation and infiltrating progenitor cells (these paradigms are examined in more detail in Archer et al. [17]). Ca = calcium; Th2 = t-helper lymphocytes; Treg = T regulatory cells.

epigenetic control of additional key genes, are required to verify their contribution to the pathogenesis of PH.

Apart from major effects of rare sequence variants in heritable forms of PAH, interindividual differences in response to the same stimulus are well documented in subjects exposed to environmental hypoxia at high altitude or in the context of high pulmonary blood flow or pulmonary venous hypertension. This variable expressivity most certainly involves the impact of unknown genetic influences regulating the pulmonary vascular response; this variation in response to hypoxia is also seen in different strains of rat (33) and bovines exposed to high altitude (34). Elegant studies demonstrated that this response is inherited (35), though the identification of the genetic basis is the subject of ongoing research (36).

The variable expressivity of PH might also be due to exposure to different environmental influences or comorbidities including: inflammation, auto-immunity, viral infection, and hormonal mediators. The specific topic of inflammation is addressed in the following text. Furthermore, accumulating evidence indicates that metabolic dysfunction may also contribute to variability in susceptibility and expressivity of PH (Fig. 2).

Although in general, animal models of PH do not recapitulate the severe pathology of human disease, these experimental models support the idea that PH can be triggered by different stimuli and that the structural changes observed in the pulmonary vasculature vary depending on the nature of the injurious stimulus (37). Several studies have underlined the importance of different signaling pathways in PH, including elastase inhibitors (38), antagonists of epidermal growth factor (4), or dietary copper restriction (39). The lack of animal model fidelity to the human pathology needs to be critically analyzed. The obliterative changes in the most distal pre-capillary arteries in the SU5416/hypoxia rat model (37,40), which evolves over time (40), do not fully recapitulate the large plexogenic lesions seen in patients—its potential reversibility may also indicate the model may not be fully reflective of the largely irreversible nature of the human disease. Indeed, recent studies indicate that the long telomeres present in mice may limit the extent to which animal model can phenocopy human disease.

Several genetic, molecular, biochemical, and environmental factors may explain the variable expressivity of PH and may contribute to its aggravation or acceleration. Severe PAH seems to represent the far end of a spectrum, in which there is an augmented burden of vascular injury and dysregulated repair (Fig. 1). Further studies, which likely require a "systems biology-like" approach, are needed to establish which key signaling pathways in pulmonary vascular cells lead to severe disease. Factors that contribute to this accelerated pathology include genetic abnormalities that may affect: 1) the exuberance of the cellular response; 2) the chronicity of an inflammatory stimulus; and 3) the severity of the altered metabolic state and cumulative DNA damage.

What Are the Differences and Similarities Between Cell Proliferations in PAH When Compared With Traditional Neoplastic Disease?

The concept of a neoplastic-like pathobiology of PH (in particular in PAH) (41) has its origins in the finding that endothelial cells in IPAH plexiform lesions are clonal when compared with similar lesions in lungs of patients with congenital heart disease (42), coupled with somatic instability in PAH lesions (29) and cultured lung endothelial cells (30). Similar to the concept of an uncontrolled cell growth in PAH,

early data emphasized the up-regulation of HIF-1α in plexiform lesions and pulmonary arteries in PAH (43) and survivin in IPAH (44). These findings, largely centered on the angle of disorganized cell growth, favored the emergence of an apoptosis-resistant phenotype, a selection process that characterizes neoplastic processes (45). In the past 8 years, further experimental evidence emerged of metabolic plasticity and altered cell energetics in the pathogenesis of PAH (46,47). Metabolic reprogramming (Fig. 2), along with genomic instability and mutations, immune escape, and cell-growth promoting inflammation, is one of the so-called "emerging hallmarks" pertinent to the pathogenesis of cancer (48). Recent studies have delineated that cancer cells use these hallmarks as driving forces leading to cell growth and co-option of the surrounding stroma for their aggressiveness (49).

Supporting documentation of metabolic adaption of pulmonary vascular cells derived from studies in rat smooth muscle cells from the fawn-hooded model of PH (46) and cultured human IPAH cells (31). In both experimental settings, the hypertensive vascular cells would preferentially use aerobic glycolysis (instead of mitochondrial metabolism). The preferential use of aerobic glycolysis by proliferating cells (including cancer cells) would endow a selective growth advantage to pulmonary vascular cells: glucose and glycolytic intermediates provide key substrates for the pentose phosphate shunt to generate reducing equivalents in the form of reduced nicotinamide adenine dinucleotide phosphate and nucleotides, essential for cell growth (50).

The preferential utilization of aerobic glycolysis appears to be related to up-regulation of several glycolytic genes and increased expression of the subunit 4.2 of cytochrome oxidase in the mitochondrial respiratory chain, all events driven by expression of HIF-1α (51). In human PAH lungs, HIF-1α appears to be expressed by endothelial cells (43) and smooth muscle cells (46). The source of stabilization of HIF-1α remains unclear, but it appears to be related to altered cellular oxidant/antioxidant balance (46,52) present in hypertensive cells. Moreover, HIF-1α could also account for the increased mobilization of hematopoietic precursors in PAH (53–55), which might promote further injury to the pulmonary endothelium (13) and possibly to contribute to the remodeled cell population (Fig. 2).

In addition to playing roles in the metabolic adaption and mobilization of bone marrow precursors, HIF-1α also participates in the regulation of mitochondria dynamics. HIF-1α accounted for decreased numbers of mitochondria and decreased nitric oxide availability in IPAH cells when compared with control cells (43); of interest, these alterations also correlate with increased IPAH endothelial cell proliferation when compared with normal endothelial cells in culture (56). HIF-1α–dependent mitochondria plasticity also pertains to PH smooth muscle cells. Activation of HIF-1α activity with cobalt or desferrioxamine leads to mitochondria fission (mediated by activation of dynamin related protein-1) in human PAH and rodent PH smooth muscle cells; this is driven by activation of dynamin-related protein-1 by active cyclin B1 (57). Finally, consistent with the role of mitochondria fission in PH smooth muscle cell growth, inhibition of dynamin-related protein-1 with the peptide Mdiv-1 blocks cell proliferation in cultured cells as well as PH caused by in vivo administration of cobalt and chronic hypoxia (57). Conversely, overexpression of mitofusin-1, which leads to mitochondria fusion, counteracts the mitochondria phenotype in PH smooth muscle cells (58).

The paradigm of metabolic plasticity and adaption of PH cells has wider implications, interfacing with controls of cellular stresses and protein misfolding and protein sorting in cytoplasmic compartments (59). In line with these paradigms, there is evidence of an altered endoplasmic reticulum (ER) stress response in PH (60), which may ultimately contribute to both adverse remodeling and elevation of pulmonary artery pressures (61). Moreover, ER stress can lead to activation of the stress-related kinase activating transcription factor-6, which increases the expression of Nogo-B. This contributes to an abnormal distancing and signaling coupling (via calcium fluxes) between the ER and the mitochondria (62). These events would lead to an increase in glycolysis, favoring vascular cell proliferation and ultimately PH.

The inroads made by the metabolic-related studies in PH are starting to bear translational fruits. There is increased metabolic labeling of glucose uptake with 18-fluorodeoxyglucose of IPAH lungs and right ventricle when compared with controls (31,63), which has also been observed in animal models of PH (64,65). Pharmacologic targeting of the preferential use of glycolysis by PH cells is being tested in humans with the small molecule dichloroacetate (NCT01083524), which improves PH in animals by blocking pyruvate dehydrogenase kinase, a negative regulator of pyruvate import into the mitochondria (66).

We are at the beginning of deciphering the role of cellular metabolism in the pathogenesis of PH and how this fundamental process relates to inflammation, remodeling, therapeutic response, and so on. Genetic modifications that favor glycolysis in multiplying cells would certainly contribute to part of the process of remodeling. However, many of the vascular cells in established human PH are quiescent, long-lived, and probably resistant to apoptosis. These quiescent cells would have substantially different metabolic requirements from those of proliferating cells. Clarifying this metabolic heterogeneity and how it contributes to remodeling and vasoconstriction will be critical moving forward. The interface of cell metabolism with the genetic basis of PAH and inflammation will certainly constitute key areas of future investigation.

What Is the Role of Inflammation in the Initiation and Progression of Different PAH Types?

Inflammation has been long recognized as an important pathogenetic element in PH (67). Expanding on prior observations of accumulation of inflammatory cells in PAH, it was documented for the first time that the amount of perivascular inflammatory infiltrate, largely of lymphocytes,

correlated with parameters of pulmonary vascular remodeling and hemodynamics in PAH (15). Inflammation consists of a complex series of interactions among soluble factors and immunologically specialized cells triggered in response to traumatic, infectious, post-ischemic, toxic, or autoimmune injury (68). It is increasingly clear that early and persistent inflammation is present and contributes to pulmonary vascular disease, as documented in tissue-based studies and studies of circulating inflammatory cells and chemical mediators.

The presence of elevated levels of circulating inflammatory cytokines, such as interleukin-1α, in PAH is well recognized (69). More recently, a wide range of cytokines has been shown to be elevated and to correlate with survival in PAH (70). At the tissue level, traditional cellular components of inflammation are described in the hypertensive pulmonary circulation (71,72). More specifically, cellular inflammation involves increases in the number of perivascular macrophages (CD68+), macrophages/monocytes (CD14+), mast cells, dendritic cells (DCs) (CD209+), T cells (CD3+), cytotoxic T cells (CD8+), and helper T cells (CD4+) in the walls of PAH vessels compared with control subjects (73). FoxP3(+) cells have been shown to be significantly decreased, suggesting a reduction in the local number of T regulatory (Treg) cells (73). Although increased numbers of circulating Treg cells has been reported in patients with idiopathic PAH (74,75), the reduced numbers of Treg in lung tissue may reflect decreased tissue recruitment of these cells. Given their immune soothing role, a diminished negative regulation on other active immune/inflammatory cells may trigger or amplify pulmonary vascular remodeling and PH.

The innate inflammatory system appears to also participate in PAH. Natural killer cells, which target stressed, virally infected, or oncogenically transformed cells independent of antigen recognition, are dysfunctional, with reduced number and cytolytic capacity, in patients with idiopathic PAH and in mouse and rat models of PH (76). Neutrophils may have potentially unrecognized roles as sources of powerful proteases, including elastases, which have recognized roles in pulmonary vascular remodeling (38,77). Because hypoxia exerts profound effects on neutrophil function (e.g., resistance to apoptosis [78]), further evaluation of the role of the neutrophil in PAH is overdue. The complement system, which bridges innate and adaptive immunity, is also activated in PAH, and deficiency of complement C3 protects mice from hypoxia-induced PH (79). Mechanistic studies focusing on the innate immune system are needed for a better appreciation of its role in PH.

However, recent insights highlighted a potential role for acquired immunity, suggesting that the pathogenesis of PH may involve specific and targeted immune cellular responses. A recent study identified a large number of tertiary lymphoid follicles in lungs from patients with idiopathic PAH when compared with control lungs (80). These lymphoid follicles in idiopathic PAH patients displayed the canonical cellularity and structure of *bona fide* tertiary follicles, whereas lymphoid organogenic chemokines, such as CXCL13 and CCL19/CCL21, were overexpressed. These data provide a structural basis for a local autoimmune response in this disease (81–83). Finally, inflammation is closely associated with pulmonary vascular disease in the setting of autoimmune diseases, such as scleroderma, and paradigmatic of the interplay of inflammation and PH is its link with the infection with the parasite *Schistosoma mansoni* (the most frequent cause of PAH worldwide [84]) and human immunodeficiency virus infection (85).

Vascular inflammation in general and PH in particular has traditionally been considered an "inside-out" response centered on leukocyte/monocyte recruitment to the intima of blood vessels driven by the endothelium-expressed adhesion molecules. Growing experimental evidence, however, supports an alternative paradigm of an "outside-in" hypothesis, in which vascular inflammation is initiated in the adventitia and progresses inward toward the media and intima, coupled with the activation of the remodeling process. In support of the "outside-in" hypothesis of adventitial regulation of inflammation are observations that, in a wide variety of vascular injuries, including PH, there is a rapid influx of leukocytes into the adventitial compartment (86). The immediate perivascular environment in PH becomes populated by inflammatory/progenitor cells, in a niche dominated by adventitia fibroblasts (37,71,86–90). These adventitial fibroblasts and recruited monocytes, particularly under hypoxia, express in a time-dependent and pulmonary artery–specific manner several cytokines/chemokines, their receptors, and adhesion molecules; these cells, therefore, appear to initiate and perpetuate the inflammatory response in an "outside-in" fashion, possibly mediated by nuclear factor kappa B signaling (91,92). Therefore, temporal-spatial dysregulation and/or failure in the normal "switch-off signal" in fibroblasts and/or macrophages/DCs may directly contribute to the persistence of a chronic inflammatory immune response. These adventitia processes are more evident in nonmouse models and in the lungs of patients with PH, possibly due to the presence of bronchial circulation.

The action of secreted cytokines, including TGF-β, in the adventitia mediates specific homing for leukocytes in this vascular compartment, leading to their inappropriate/pathologic retention and survival (92). These homing/recruitment cytokines include up-regulated adhesion molecules, including intercellular adhesion molecule 1 and vascular adhesion molecule-1, and stromal derived factor-1/CXCR4, which promotes adhesion of leukocytes and bone marrow precursor cells, respectively. The adventitia is, therefore, suited to harbor canonical innate immune cells, specifically macrophages and DCs, which with adventitial fibroblasts, are all equipped with the necessary machinery (e.g., toll-like receptors and inflammasome components [like the nod-like receptors]) to potently respond to a variety of exogenous and endogenous danger signals.

Importantly, there is growing evidence that epigenetic marks may "lock" innate immune cells into a distinct functional phenotype, with loss of functional plasticity and failure to respond to regulatory signals. The inflammatory microenvironment in the pulmonary circulation promotes epigenetic marks in macrophages, locking their functional phenotype into a pro-fibrogenic and pro-remodeling macrophage (93–97). Different signaling processes, mediated largely by signal transducers and activators of transcription 1, 3, and 6, may drive macrophage-promoted remodeling (98); it is likely that all of these macrophages are present in PH and play complex and time-dependent, sometimes overlapping or dedicated, roles in the disease. Moreover, it is also likely that individual macrophages are plastic, changing their function and signaling according to the disease stage and specific molecular triggers.

Finally, emerging concepts involve the potential of organ-specific microbiota and their metabolic products to engage the inflammasome pathways and to influence pulmonary vascular responses, as shown in the liver (99). A further pathway by which remote signaling might target the pulmonary vasculature is by the release of cell-derived exosomes from distant sources, exerting anti- or pro-inflammatory depending on their cargo. Exosomes derived from mesenchymal stem cells were found to inhibit the hypoxic activation of the signal transducers and activators of transcription 3 pathway in hypoxic mice and prevent the development of PH (100).

Conclusions

Cytokines and immune cells appear to play significant but complex roles in the initiation and progression of PH. Major unanswered questions include:

1. What determines the abnormal host response to inflammation that leads to the initiation and progression of PH?
2. Is the inflammatory response in PAH caused by autoimmunity or infection?
3. Which specific features of the inflammatory response can be enhanced (if protective) or blocked (if detrimental) for therapeutic intervention?

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